Here a novel methodology is described where bovine IgG may be very rapidly removed from serum preparations for use in cell culture using immunoaffinity columns in Perfusion Chromatography (Afeyan et al., 1991). Perfusion chromatography involves the flow of liquid through a non-compressible porous chromatographic particle (POROS® Media, PerSeptive Biosystems) with 6000-8000 A pores which transect the particle. These through pores allow very high flow rates and enable rapid loading, washing cleaning and elution of the column. We have applied perfusion affinity-chromatography using the BioCAD® Workstation.

Please replace the first full paragraph on page 5 with the following replacement paragraph:

Development and modernization of the methodologies of purification and isolation of protein molecules are needed to keep pace with the state-of-the-art technologies for protein purification and peptide analyses. The methodology described above serves as a model for the rapid purification of all other MHC class I and class II molecules. The speed of purification reduces the handling time of the serum preparation and ensures an improved cost effectiveness and quality. Such a technological advance is fundamental to a sophisticated study of the immune response to foreign antigens, self-tolerance and autoimmunity and to the development of peptide vaccines based on the use of MHC-restricted epitopes for anti-tumor and anti-viral immunotherapy.

Please replace the second full paragraph on page 5 with the following replacement paragraph:

Pure monoclonal antibodies, humanized antibodies and Fc-fusion (chimeric) proteins are finding increased use in therapeutics and biomedical research. One of the more commercially popular ways is to produce antibodies as cell culture supernatant followed by affinity purification. Most antibody producing cells require serum-containing medium. This medium preparation has antibodies from the serum component. The spent medium thus has the antibody of interest and the

previously present antibodies. The prior art technique of separating an antibody of interest from the previously present antibodies is expensive and technologically involved. However, using this invention, a medium free of antibodies could be generated for the culture of antibody producing cells in a cost efficient manner.

Please replace the first full paragraph on page 6 (after the heading Production and Purification of Monoclonal Antibodies) with the following replacement paragraph:

The above medium was used to grow the monoclonal antibody producing hybridoma cell line ME1 (an anti-HLA-B27 mouse IgG1 monoclonal antibody (Ellis et al., 1982), LB3.1 (an anti-HLA-DR, mouse IgG2b monoclonal antibody (Gorga et al., 1986) and 4418 (an anti NKp44, mouse IgG1 monoclonal antibody). The LB3.1 monoclonal antibody was purified by running the cell culture supernatant on POROS® 20A (protein A coupled POROS® 20 medium) and the ME1 and 4418 monoclonal antibodies were purified by running the cell culture supernatant on POROS® 20G protein G coupled to POROS 20® medium used for IgG1 antibodies) column using a BioCAD™ Workstation for perfusion chromatography (PerSeptive Biosystems). Typically 1-2 liters of cell culture supernatant was filtered through 0.2 micron filter and run on a POROS® 20A or a POROS® 20G column. The column was washed with 5 column volume of 2% acetic acid. The eluted antibody was immediately neutralized with 1M Tris base and the column equilibrated with PBS.

Please replace the second full paragraph on page 6 (after the heading Production and Purification of a Fc fusion protein exemplified by NKp46-Ig) with the following replacement paragraph:

Transiently transfected COS cells with NK-46Ig gene construct were grown in the IgG free medium. The supernatant from these cells was run on POROS® 20G column as above and the pure NKp46-Ig fusion protein purified.

Please replace the paragraph bridging pages 6 and 7 (after the heading Preparation of Immunoaffinity Columns for removal of serum proteins from serum containing media prior to preparation of cell culture products) with the following replacement paragraph:

Typically 10-20 mg of the purified monoclonal antibody in PBS was coupled to one ml of POROS® 20 AL medium (POROS® 20 medium activated with the aldehyde group)(PE Biosystems). To about 5-10 mg/ml of antibody in PBS was added ½ volume of High Salt Buffer Solution (1.5 M sodium sulfate in 100 mM sodium phosphate 7.4). This was made 5-10 mg/ml in NaCNBH3 (Sigma). To this was added the appropriate amount of POROS® 20 AL (generally slightly more than the desired column volume) and the solution was made to 0.9-1.1 M in Na2SO4 by the addition of High Salt Buffer Solution. The final concentration of the antibody was between 1-2 mg/ml. The reaction was carried out overnight by gentle shaking. The media was filtered in a 10-20 m sintered glass funnel and resuspended in 50-100 ml of Capping Buffer (5 g/l NaCNBH3 in 0.2 M Tris, pH 7.2) for about one hour. The media was then washed with PBS and packed in a column. Columns ranging from 4.4 ml (100x7.5 mm) to 13.25 ml (300x7.5 mm) PEEK (polyetheretherketone) columns (Alltech) were packed under the conditions specified by the manufacturer. A pre-clearing column using normal mouse serum (NMS) was also prepared and used to remove proteins that adhered non-specifically to IgG.

Please replace the first paragraph on page 9 (after the heading Table 2) with the following replacement paragraph: